Corticosterone levels in relation to trace element contamination along an urbanization gradient in the common blackbird (*Turdus merula*)

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Abstract: In a rapidly urbanizing world, trace element pollution may represent a threat to human health and wildlife, and it is therefore crucial to assess both exposition levels and associated effects of trace element contamination on urban vertebrates. In this study, we investigated the impact of urbanization on trace element contamination and stress physiology in a wild bird species, the common blackbird (*Turdus merula*), along an urbanization gradient (from rural to moderately urbanized areas). Specifically, we described the contamination levels of blackbirds by 4 non-essential (Ag, Cd, Hg, Pb) and 9 essential trace elements (As, Co, Cr, Cu, Fe, Mn, Ni, Se, Zn), and explored the putative disrupting effects of the non-essential element contamination on corticosterone levels (a hormonal proxy for environmental challenges). We found that non-essential trace element burden (Cd and Pb specifically) increased with increasing urbanization, indicating a significant trace element contamination even in medium sized cities and suburban areas. Interestingly, the increased feather non-essential trace element concentrations were also associated with elevated feather corticosterone levels, suggesting that urbanization probably constrains birds and that this effect may be mediated by trace element contamination. Future experimental studies are now required to disentangle the influence of multiple urban-related constraints on corticosterone levels and to specifically test the influence of each of these trace elements on corticosterone secretion.

Keywords: urban environments; trace metals; stress physiology; endocrine disruptor; passerine bird

1. Introduction

Anthropogenic activities have continuously released a wide variety of pollutants into the environment (Azimi et al., 2003, 2005; Pacyna and Pacyna, 2001; Van der Gon et al., 2007), and the worldwide contamination of ecosystems has rapidly become of environmental concern (Carson, 1962; Colborn et al., 1993; Mergler et al., 2007; Peakall, 1992). Due to the constant expanding urbanization and associated industrial processes, trace elements in particular, can be a major environmental issue in urbanized environments (Azimi et al., 2005; Nam and Lee, 2006; Roux and Marra, 2007; Scheifler et al., 2006; Wei and Yang, 2010). Despite their natural origin (Nriagu, 1989), trace element emissions have been significantly increased by anthropogenic activities (e.g., mining, fossil fuels combustion, waste products of industrial activities; Azimi et al., 2005; Nriagu, 1990; Selin, 2009; Walker et al., 2012). These contaminants are particularly persistent and toxic (especially the non-essential elements such as cadmium (Cd), mercury (Hg), and lead (Pb); Domingo, 1994; Scheuhammer, 1987; Walker et al., 2012; Wolfe et al., 1998), and importantly, they can bio-accumulate in organisms and, for some elements, bio-magnify through the food chains (Walker et al., 2012). Increased trace element pollution in urban environments may thus represent a threat for human health and wildlife, and it is therefore imperative to accurately assess exposition levels and potential associated detrimental effects of these pollutants on urban vertebrates.

Wild animals have successfully and increasingly been used as biomonitors of trace element contamination in the past decades (Alleva et al., 2006; Burger, 1993; Furness, 1993; Lodenius and Solonen, 2013). Because of their wide distribution and their often high trophic levels, birds have especially been proposed as suitable indicators (Burger, 1993; Furness, 1993), and therefore, the determination of trace element concentrations in various tissues of bird species (e.g., blood, feathers, liver, kidney, muscle, eggs, feces) has been widely used in biomonitoring studies (e.g., see Berglund et al., 2015; Burger and Gochfeld, 1992; Carravieri et al., 2014a; Costa et al., 2014; Dauwe et al., 2000; Eens et al., 1999; Frantz et al., 2012; Orłowski et al., 2015; Swaileh and Sansur, 2006). However, most studies assessing the exposure of birds to trace element pollution have focused on heavily contaminated areas (e.g., vicinity of smelters; see Berglund et al., 2011; Coeurdassier et al., 2012; Dauwe et al., 2000, 2004; Janssens et al., 2002) or aquatic ecosystems (e.g., see Burger and Gochfeld, 2004; Carravieri et al., 2014b; Furness and Camphuysen, 1997; Hernández et al., 1999; Kalisińska et al., 2004), and comparatively, moderately polluted urban and suburban environments have been less studied (but see Costa et al., 2013; Frantz et al., 2012; Roux and Marra, 2007; Scheifler et al., 2006; Torres et al., 2010; Swaileh and Sansur, 2006) despite their ecological importance (Grimm et al., 2008). Importantly, trace element contamination has been associated with reduced breeding performances, reduced survival, and therefore poor individual fitness (Brasso and Cristol, 2008; Goutte et al., 2014; Hallinger et al., 2011; Scheuhammer et al., 2007; Varian-Ramos et al., 2014; Wolfe et al., 1998), even when contamination was far below a lethal threshold. At low or moderate doses, the detrimental effects of trace elements are thought to be primarily mediated by alteration and disruption of central physiological and behavioural mechanisms that govern the seasonal and daily routines of wild vertebrates (Wingfield, 2008). For instance, accumulation of trace elements has been associated with immunosuppression and prevalence of infectious disease (Bichet et al., 2013; Gasparini et al., 2014; Hawley et al., 2009; Snoeijs et al., 2004), altered behaviour and reproductive impairment (Evers et al., 2008; Frederick and Jayasena, 2010; Janssens et al., 2003; Tartu et al., 2013, 2015), and nutritional stress (Eeva et al., 2000, 2003). Trace element contamination can therefore disrupt homeostasis and can represent a stressful challenge for wild vertebrates.

In that respect, measuring glucocorticoid levels is useful and relevant to assess to what extent trace element contamination might affect wild vertebrates in urban environments. Glucocorticoids are one of the main mediators of allostasis in vertebrates (McEwen and Wingfield, 2010; Romero et al., 2009) and slight increases in circulating corticosterone levels (hereafter CORT, the main avian glucocorticoid) aim at restoring homeostasis when energetic challenges occur (Angelier and Wingfield, 2013; Landys et al., 2006; Romero, 2004). Therefore, elevated CORT levels are classically viewed as a reliable proxy for a high allostatic state, and thus, for important energetic constraints (Angelier and Wingfield, 2013; McEwen and Wingfield, 2010; Romero et al., 2009). In addition, there is also important evidence, mostly from laboratory studies, that non-essential trace elements (e.g., Cd, Hg and Pb) can act as powerful endocrine disruptors and result in abnormal or modified circulating hormone levels, even at very low concentrations (Colborn, 2004; Giesy et al., 2003; Ottinger et al., 2005; Tan et al., 2009). The disruption of CORT regulation may prevent individuals from restoring homeostasis when environmental challenges occur, and such endocrine disruption could therefore be a major cause of reduced performances in wild vertebrates. However, the relationship between trace element contamination and glucocorticoid levels in wild vertebrates needs further attention. Indeed, most studies have been limited to a single contaminant or to elevated doses of contaminants (Franceschini et al., 2009; Heath et al., 2005; Herring et al., 2012; Tartu et al., 2013, 2015; Wada et al., 2009) and, thus far, no clear patterns of the effects of trace elements on CORT concentrations have been revealed.

In this study, we investigated the impact of urbanization on trace element contamination and CORT levels in a wild bird species, the common blackbird (*Turdus merula*). Specifically, we sampled breast feathers of 44 adult and juvenile blackbirds to concomitantly measure feather trace element concentrations (Burger, 1993) and feather CORT levels (Bortolotti et al., 2008). So far, most studies have focused on large and dense cities (e.g., see Roux and Marra, 2007) while overlooking moderately urbanized cities (i.e., less densely populated, with lower building density and higher vegetation coverage) that, yet, represent a large and increasing part of urbanized land cover (United

Nations, 2015). Our first goal was therefore to document the contamination of blackbirds by 4 toxic non-essential trace elements (silver (Ag), Cd, Hg and Pb) and 9 essential trace elements (for which only high levels can be toxic: arsenic (As), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se) and zinc (Zn)) along an urbanization gradient (from rural to moderately urbanized areas) in France. Because urbanized areas are characterized by important anthropogenic activities, we logically predicted that trace element contamination of blackbirds will increase along the urbanization gradient. We also predicted that trace element contamination will remain moderate because of limited anthropogenic activities in medium-sized cities. Second, we focused on the non-essential trace elements (Ag, Cd, Hg and Pb) because they are known to be highly toxic and to have endocrine disrupting properties even at very low concentrations (Colborn, 2004; Giesy et al., 2003; Ottinger et al., 2005; Tan et al., 2009). Our second goalwas therefore to test whether this non-essential trace element contamination is associated with modified CORT levels. For instance, if trace element contamination energetically constrains blackbirds, we predicted that trace element levels will be positively correlated with CORT levels as elevated CORT levels are usually related to high allostatic load and important energetic constraints (*sensu* McEwen and Wingfield, 2010). Alternatively, if these trace elements act as endocrine disruptors, it may also result in abnormal reduced CORT levels as previously suggested in other studies (e.g., see Wada et al., 2009).

2. Materials and Methods

2.1. Study area and sample collection

A total of 44 blackbirds (31 adult males and 13 juveniles) were sampled between January 2013 and August 2014 along an urbanization gradient in the Poitou-Charentes region, Western France.

Among them, 32 blackbirds were found dead (from vehicle collisions), collected and stored in metal-free polyethylene bags at −20 °C, while the 12 others were opportunistically captured with mist-nets and released immediately after feather sampling. For each individual, a few breast feathers (up to 10–12 feathers for live individuals) were collected and stored dry in metal-free polyethylene bags until analyses. Birds were aged as juvenile (i.e., first year individual before the post-juvenile molt) or adult based on plumage characteristics (Cramp, 1988). In blackbird, juveniles and adult males are predominantly victims of road traffic (Erritzoe et al., 2003). Thus, to avoid an unbalanced adult sex-ratio (only 3 females were collected) and potential gender-related differences in trace element contamination (e.g., due to diets differences between males and females or due to the ability of females to eliminate part of their trace element burden in their eggs; Burger, 2007), adult females were not included in this study. Conversely, we could not determine the sex of the juveniles and, thus, both male and female were probably analyzed. However, because the feathers of juveniles have grown during their development in the nest (and thus, while both sexes share the same environment and the same diet), differences in trace element contamination between genders are much less likely to occur.

Blackbirds were sampled in locations that differ in urbanization rates (i.e., urban and suburban areas, and rural areas surrounded by agricultural areas or forests). For each bird, the geographic coordinates of the site of collection were recorded (see Supplementary Material, Table A.1 in the online version at http://dx.doi.org/10.1016/j.scitotenv.2016.05.014). To quantify the degree of urbanization of each location, we used a slightly modified version of the method described in Bichet et al. (2013). Briefly, we acquired habitat characteristics from the CORINE Land Cover database (CLC2006; see http://www.eea.europa.eu/publications/COR0-landcover) using the open source geographic information system QGIS (QGIS 2.4.0; www.qgis.org). We used a circle of 3.5 km radius centered on the sampling location (because, mean adult and natal dispersal distances of blackbirds are around 3.3 km; Paradis et al., 1998) and extracted the surfaces (km^2) of five variables that describe the major habitat characteristics (Table A.1 in the online version at http://dx.doi.org/10.1016/j.scitotenv.2016.05.014): (1) urban areas (urban fabric and industrial, commercial and transport units), (2) arable lands, (3) pastures, (4) woodlands, and (5) scrublands. We then calculated the PC1 value from a principal component analysis (PCA) on the 5 variables of land cover (log-transformed) for each location. The PC1 accounted for 67.6% of the total variance, and correlated positively with urban areas (r = 0.85) and negatively with forests (r = −0.91) and scrublands (r = −0.90). Areas with PC1 value around 0 corresponded to agricultural areas. The PC1 values were thus used as an "urbanization gradient" (Table A.1 in the online version at http://dx.doi.org/10.1016/j.scitotenv.2016.05.014).

In this study, we used two sampling methods: collection of carcasses and capture of live individuals. Because fewer blackbirds were found dead in urban areas compared to rural areas (urban: 7 individuals; rural: 25 individuals),we also captured individuals at sites located within two medium-size cities (Niort and La Rochelle; urban: 12 individuals; Table A.1 in the online version at http://dx.doi.org/10.1016/j.scitotenv.2016.05.014). The birds caught alive were captured close to locations where birds road-killed had been found, using mist-nets positioned along a road. Importantly, there were no effect of the sampling method on all parameters investigated (i.e., trace element levels and CORT levels; see Section 2.4. for details).

2.2. Sample preparation and trace element analyses

Prior to trace element analyses, feathers were washed in a chloroform-methanol solution to remove adsorbed external contamination and dirt, and then oven dried (48 h, 50 °C) to constant dry mass as described in Blévin et al. (2013). For each individual, washed feathers were then pooled and homogenized by crushing them to powder with scissors, and then stored in plastic tubes. For 35 individuals, 2 feathers were kept unwashed for corticosterone analyses (see Section 2.3 for details). Trace elements were determined in the washed feather samples at the University of La Rochelle (LIENSs), France. First, total Hg (hereafter Hg) concentrations were measured using an Advanced Mercury Analyzer spectrophotometer (Altec AMA 254) on dried feather aliquots (5–10 mg) following Blévin et al. (2013). For each sample, analyses were run 2–3 times until having a relative standard deviation (RSD) b 10%. Blanks were analyzed at the beginning of each set of samples. The limit of detection (LoD) of the method was 0.005 $\mu g \cdot g^{-1}$ dry weight (dw). Measurements quality was assessed using a certified reference material (TORT-2 Lobster Hepatopancreas, National Research Council, Canada: certified Hg concentration: 0.27 ± 0.06 $\mu g \cdot g^{-1}$ dw). Our measured values were 0.268 ± 0.018 $\mu g \cdot g^{-1}$ dw (n =13).

Second, 12 other trace elements (Ag, As, Cd, Co, Cr, Cu, Fe,Mn, Ni, Pb, Se and Zn)were measured using a Varian Vista-Pro ICP-OES and a Thermo Fisher Scientific X Series 2 ICP-MS following Metian et al. (2008). Aliquots of the dried feather samples (20–200 mg; weighted to the nearest 0.1 mg) were digested in a mixture of 6 ml of 65% HNO_3 (VWR Quality SUPRAPUR) and 2 ml of 30% HCl (VWR Quality SUPRAPUR), except for the samples with a weight below 0.1 g (3 ml HNO_3 and 1 ml HCl). Acidic digestion of the samples was carried out overnight at room temperature and then using a Milestone microwave (30 min with constantly increasing temperature up to 120 °C, and then 15 min at this maximal temperature). After digestion, samples were diluted to 50 ml (25ml for the samples below 0.1 g) with ultrapure water. To avoid trace element contamination, all glass and plastic utensils used were soaked in a bath of nitric acid (50ml in 2 l) for a minimum of 48 h, rinsed in ultrapure water and dried under a laminar hood before use. Accuracy and reproducibility of the preparation were tested by preparing analytical blanks and replicates of Lobster Hepatopancreas (TORT-2) and Dogfish Liver (DOLT-4) certified reference materials (NRC, Canada) along with each set of samples. Results for the certified reference materials were in good agreement with the certified values and recovery rates varied from 83% to 115% for TORT-2 and 84% to 118% for DOLT-4. The LoD ($\mu g \cdot g^{-1}$ dw) were 0.04 for Ag, Cd, Co, Cr, and Pb, 0.07 for Ni, 0.19 for Mn and Se, 0.37 for As, 1.85 for Cu, and 7.41 for Fe and Zn (calculated using blank values and average dry mass of samples). Trace element concentrations are expressed in $\mu g \cdot g^{-1}$ on a dry weight (dw) basis.

2.3. Corticosterone analyses

Because we did not have enough feathers after trace element analyses for 9 blackbirds, only 35 blackbirds were assayed for CORT analyses. For each individual, two unwashed feathers were measured (length) with a caliper to the nearest 0.1 mm and weighed with a precision scale to the nearest 0.1 mg. All measurements were made by the same person to avoid extra variation in the data. Feather CORT concentrations were then measured following the protocol described by Bortolotti et al. (2008) with minor modifications. 10 ml of methanol (HPLC grade) was added to each feather sample to extract CORT from the feather. The samples were placed in a sonicating water bath at room temperature for 30 min, followed by incubation at 50 °C overnight in a shaking water bath. The methanol was then separated from feather material by filtration, using filtered syringes. The feather remnants, original sample vial and filtration material were washed twice with 2.5 ml of additional methanol and the washes were added to the original methanol extract. The methanol extract was placed in a 50 °C water bath and subsequently evaporated in a fume hood under air. The extract residues were reconstituted in a small volume of the phosphate buffer system (PBS; 0.05 m, pH 7.6). These feather extracts were then analyzed by radio-immunoassay at the Centre d'Etudes Biologiques de Chizé (CEBC) as previously described (Lormée et al., 2003). The intra- and inter-assay coefficients of variation were 7.79% and 8.61% respectively (samples were run in 3 assays).

2.4. Statistical analyses

All statistical analyses were performed in R 3.1.0 (R Core Team, 2014). Trace elements for which concentrations were lower than the LoD in N30% of individuals (Ag and As) were included in summary statistics but excluded from subsequent statistical analyses. For the other trace elements, concentrations below the LoD were replaced by (LoD)*0.5 and considered for further statistical analyses (EPA, 2000). Because feather samples were collected from blackbirds that were found dead but also from live individuals that were captured with mist-nets, we first ensured that feather trace element concentrations and feather CORT levels did not differed between sampling method using One-way ANOVAs. Since we did not capture birds in rural areas, we only performed these tests on individuals collected in urban and suburban areas (n = 12 captured alive and n = 7 found dead) and we found no effect of the sampling method on trace element concentrations (ANOVAs: p N 0.275 for all considered trace elements) or CORT levels (ANOVA: p = 0.719).

Second, we used linear models (LMs) to test the influence of urbanization (urbanization gradient; continuous variable), age (two-level factor: juvenile and adult) and their interaction on trace element concentrations. Trace element concentrations were log-transformed to obtain normal distribution, but we present non-transformed values in figures to facilitate interpretation. Similarly, we then used LMs to test the influence of urbanization, age and their interaction on CORT concentrations (log-transformed). Finally, for 3 non-essential elements (Cd, Hg, and Pb) we used LMs to examine the influence of feather trace element concentrations (log-transformed), age, and their interaction on feather CORT levels (log-transformed).

3. Results

3.1. Summary statistics

Concentrations of the 13 trace elements in breast feathers of adult and juvenile blackbirds are listed in Table 1. All trace elements investigated were detected in the feathers of blackbirds. Among them, only two elements (Ag and As) were below the LoD in N30% of individuals (Table 1).

3.2. Effect of urbanization on trace element concentrations

Regarding the non-essential trace elements (Cd, Hg, and Pb), both feather Cd and Pb concentrations were positively and significantly related to the degree of urbanization (Table 2, Fig. 1). In contrast, feather Hg concentrations were not related to the degree of urbanization (Table 2, Fig. 1). Feather Cd, Hg and Pb concentrations were similar between juveniles and adults (Table 2). Moreover, the "urbanization × age" interaction was never significant, suggesting that the influence of urbanization on feather Cd and Pb concentrations did not significantly differ between adults and juveniles.

For the essential trace elements, only feather Se and Zn were positively and significantly related to the degree of urbanization (Table 3). In particular, for Zn concentrations, the "urbanization × age" interaction was marginally significant (Table 3): feather Zn concentrations increased with increasing urbanization in adults only (slope estimates ($\beta \pm SE$) adults: 0.121 ± 0.044, $p = 0.008$; juveniles: 0.003 ± 0.047, $p = 0.944$). The other essential trace element (Co, Cr, Cu, Fe, Mn and Ni) concentrations were not significantly related to the degree of urbanization. Finally, feather essential trace element concentrations did not differ between age classes (Table 3), except feather Fe concentrations, with juveniles having lower Fe concentrations than adults.

3.3. Effect of urbanization on feather CORT concentrations

Feather CORT concentrations were positively and significantly related to the degree of urbanization (“urbanization” variable: F1,31=6.61, p = 0.015, slope estimates (β ± SE): 0.090 ± 0.035) and were similar between adult and juveniles (“age” variable: F1,31 = 0.01, p = 0.974). Moreover, the “urbanization × age” interaction was not significant (F1,31=2.84, p = 0.102), suggesting that the influence of urbanization on feather CORT concentrations did not significantly differ between juveniles and adults.

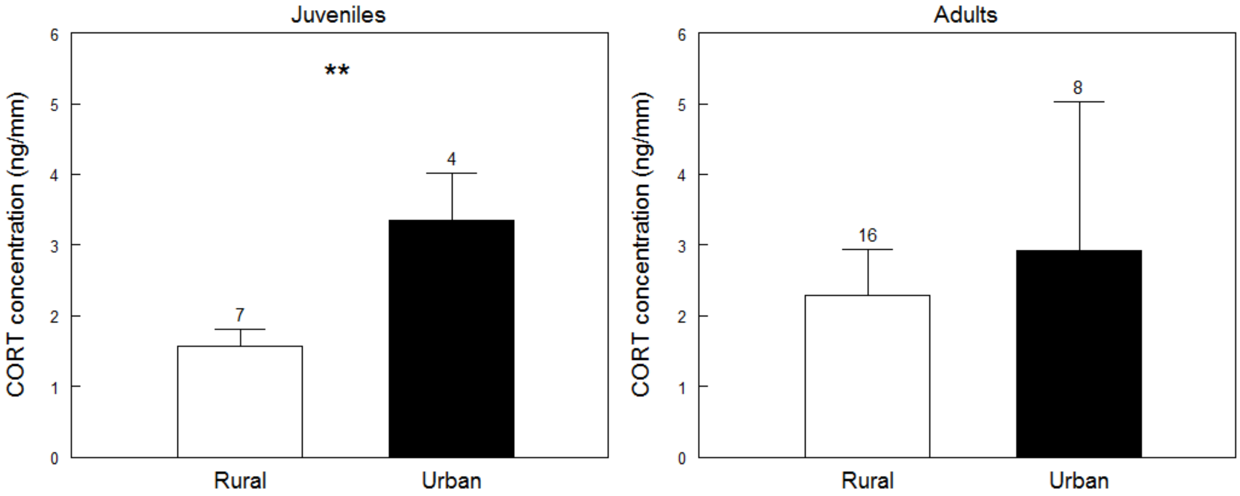
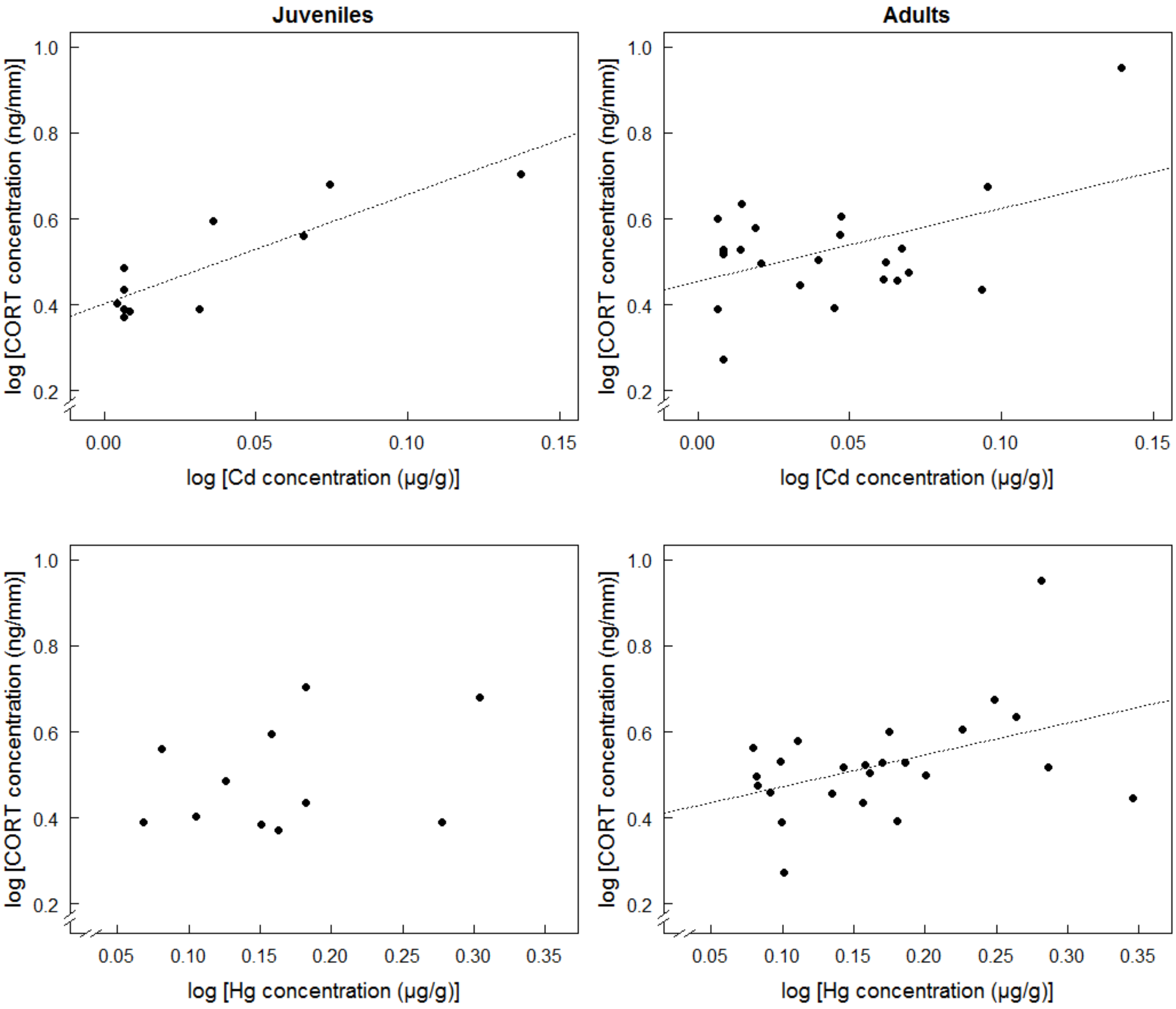


Fig 3. CORT concentrations (Mean ± SD ng.mm^{-1}) in feathers of juvenile and male adult blackbirds from rural (open bars) and urban areas (filled bars). Significant effect of habitat is symbolized: ** *p* < 0.01 (Wilcoxon test). Numbers above bars indicate sample size.

3.4. Relationships between non-essentials trace elements and CORT concentrations

Feather CORT concentrations were significantly and positively related to feather Cd, Hg, and Pb concentrations (Table 4; Fig. 2). Importantly, the “urbanization × age” interactions were not significant, demonstrating that the relationships between these non-essential trace element concentrations and CORT concentrations did not differ between adults and juveniles. When analyses were performed after removal of the individual with a very high CORT level, results were qualitatively similar. More specifically, feather CORT concentrations were positively and significantly related to feather Cd concentrations (LM, F1,30 = 10.35, p = 0.003) and feather Pb concentrations (LM, F1,30 = 13.38, p b 0.001), but only marginally significantly related to feather Hg concentrations (LM, F1,30= 3.02, p=0.092).



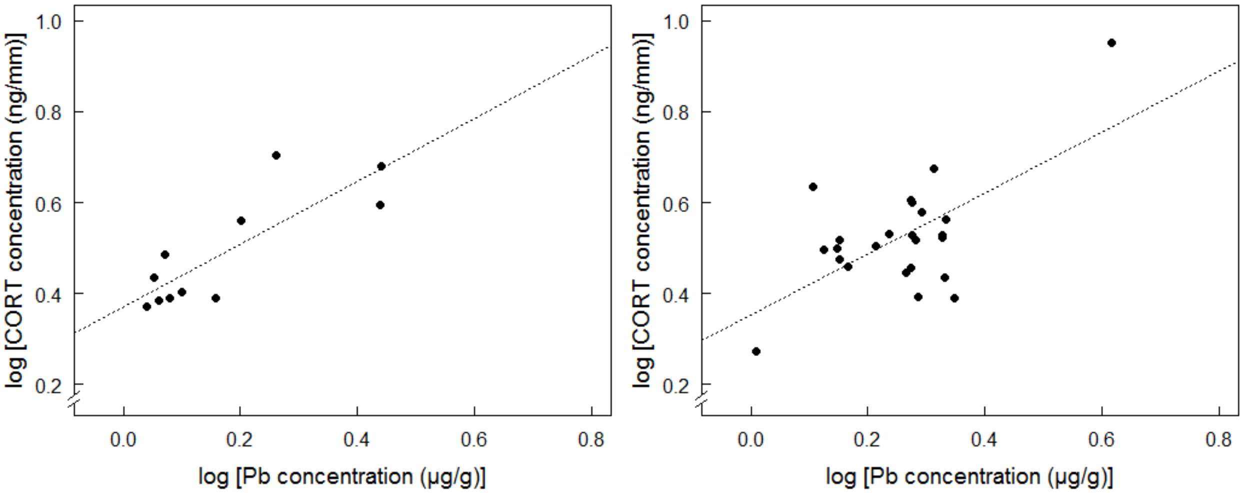


Fig 4. Relationship between feather CORT levels (log-transformed) and feather non-essential trace element levels (Cd, Hg, Pb; log-transformed) in juvenile and male adult blackbirds. Dotted lines refer to statistically significant linear regressions.

4. Discussion

In this study, we examined the influence of urbanization on trace element contamination in blackbirds along an urbanization gradient (from rural to moderately urbanized areas). We found that trace element burden (specifically the non-essential elements Cd and Pb) increased with increasing urbanization, Interestingly, the increased nonessential trace element contamination of urban birds was also associated with elevated CORT levels. This result suggests that urbanization probably energetically constrains urban birds and that this effect could be mediated, at least partly, by trace element contamination. Indeed, trace element contamination may alter the ability of individuals to cope with the urban environment, resulting therefore in elevated CORT levels. Moreover, these non-essential trace elements may also act as endocrine disruptors and affect the Hypothalamic-Pituitary-Adrenal (HPA) axis, thus explaining the observed higher CORT levels of urban blackbirds from our study. Because urbanization is not only associated with trace element contamination but also with numerous other constraints, future experimental studies are now required to disentangle the influence of these multiple urban-related constraints on CORT levels and to specifically test the influence of each of these non-essential trace elements on CORT secretion.

4.1. Urbanization and trace element contamination

Here, urbanization appears to have a significant effect on trace element contamination in blackbirds, with urban individuals having higher trace element concentrations than rural ones. Although we did not find any variation in several essential trace element concentrations (Co, Cr, Fe, Cu, Mn, and Ni) along the urbanization gradient, we found that feathers of urban blackbirds had increased Se, and Zn concentrations, and most importantly, increased non-essential trace element concentrations (Cd and Pb) compared to rural blackbirds. Importantly, our study took place in medium-sized cities and moderately urbanized areas, demonstrating therefore that such contamination is not limited to intensely urbanized areas. High blood and feather Cd and Pb burdens are often found in birds inhabiting highly urbanized and industrial areas (Bichet et al., 2013; Coeurdassier et al., 2012; Eens et al., 1999; Fritsch et al., 2012; Roux and Marra, 2007; Tête et al., 2014, but see Bichet et al., 2013; Manjula et al., 2015 for Cd). For instance, Coeurdassier et al. (2012) found that blood Cd and Pb levels reach extremely high concentrations when blackbirds were sampled on a smelter contaminated site. It is important to emphasize that the contamination levels we report here are relatively moderate or even low when compared with such studies. However, accurate comparisons between studies are difficult because trace element levels can dramatically differ among different types of feathers (Burger, 1993; Dauwe et al., 2003, see Scheifler et al., 2006 for some comparisons in the blackbird). Since the establishment of new environmental policies in the 2000′s (e.g., reduction of unleaded petrol use), Pb emissions and burdens have, respectively, decreased in urban areas and wildlife (Berglund et al., 2012; Chadwick et al., 2011). However, our study shows that this urban-related contamination remains in wild birds, even in moderately urbanized areas. Because this contamination appears persistent even after several years, trace elements can still represent an important threat for wildlife (Pouyat et al., 2015). Interestingly, we did not find any evidence of differences in trace element contamination between adult and juvenile blackbirds. Age differences in trace elements concentrations are generally expected to arise because of age-related variations in exposure (e.g., through differences in diet composition or quality, foraging behavior) or bioaccumulation with age (Berglund et al., 2011; Burger, 2008; Furness, 1993). And, contrary to our findings, age-related pattern of accumulation of Cd and Pb have been reported in blood and feathers of common blackbirds sampled along a pollution gradient near a smelter contaminated area (Fritsch et al., 2012). However, feather Cd and Pb concentrations found in the study of Fritsch et al. (2012) were among the highest reported for this species. Comparatively, the relatively low contamination levels found in medium-size cities and the moderate sample size for juveniles in the present study could have limited our ability to detect age related differences in the influence of urbanization on feather Cd and Pb contamination. On the other hand, this lack of differences could also suggest that exposure at a given site along the urbanization gradient might be quite similar for adults and juveniles during the breeding season (e.g., see Janssens et al., 2001). Indeed, feathers of juveniles are synthesized and integrate trace elements while they are developing in the nest, and similar exposure between adults and nestlings could occur if adults feed their nestlings with the same diet as their own. For instance, Pb levels in the kidneys of adult blackbirds have been shown to be comparable to Pb levels in the crop contents (diet items dominated by earthworms) of nestlings (Weyers et al., 1985). Finally, some age-related differences may also have been blurred by movements of juveniles or adults throughout the year. Blackbirds are indeed known to be either sedentary or migratory birds (Cramp, 1988). Unfortunately, in our study, we did not know whether sampled birds were migratory or not, and thus, we cannot exclude the possibility that they might have accumulated their trace element burden in another habitat than the one where they have been sampled. For the juveniles, this last interpretation is however unlikely as juvenile blackbirds are known to stay around their natal territory several weeks after fledging (Magrath, 1991). Similarly, urban adult blackbirds have been shown to be sedentary rather than migratory (Evans et al., 2012; Møller et al., 2014; Partecke and Gwinner, 2007), and dispersal is limited in male common blackbirds (Paradis et al., 1998), limiting therefore the potential of this hypothesis to explain the lack of age-related differences in trace element contamination. Moreover, our findings on the influence of urbanization on trace element contamination are consistent with the literature (higher nonessential trace element contamination in urbanized areas; e.g., see Bichet et al., 2013; Eens et al., 1999; Roux and Marra, 2007; Scheifler et al., 2006), supporting again that migratory tendency did not have a strong effect on our results.

4.2. Urbanization and feather CORT levels

We found that urbanization had a significant influence on feather CORT concentrations in blackbirds, and specifically, that CORT levels increased with increasing urbanization. This finding is supported by other studies on the endocrine ecology of urban birds (e.g., see Fokidis et al., 2009; Zhang et al., 2011), but not by others (e.g., see Atwell et al., 2012; Meillère et al., 2015). And in particular, another study on blackbirds did not find any differences in plasma baseline CORT concentrations between urban and forest birds (Partecke et al., 2006). Such discrepancies could be partly due to differences in sampling methods. Indeed, so far, most studies have focused on instantaneous measures of CORT levels (i.e., baseline and stress-induced CORT levels in the blood) that are only short-term measures of CORT levels, while we used a more-integrated measure (i.e., feather CORT levels). To date, the relationship between urbanization and CORT levels has been revealed inconsistent and probably depends on the species considered, the life-history stage, and the specific constraints of a given urban habitat (reviewed in Bonier, 2012). Birds inhabiting urban areas are exposed to numerous potentially stressful challenges (e.g., increased light, noise and chemical pollution, human disturbance, food limitation) that could explain higher CORT levels in urban areas. For instance, a previous study has shown that food limitation was more important in cities than in rural areas for nestlings blackbirds (Ibáñez-Álamo and Soler, 2010). Differences in CORT levels between urban and rural juveniles may thus result from nutritional stress when the nestlings were still growing and developing their feathers at their nest. However, this explanation seems less likely for the adults as there is no clear effect of urbanization on adult blackbirds' body condition (i.e., body mass; see Evans et al., 2009). Thus, other potential stressful factors associated with urban life (pollutions, in particular) could play a role in the increased CORT levels in urban environments.

4.3. Trace element contamination and feather CORT levels

In wild vertebrates, it is often challenging to link physiological measures and trace element contamination because most physiological parameters can only be measured in the blood that only represents an instantaneous physiological measurement. For instance, CORT fluctuates daily and seasonally (Landys et al., 2006; Rich and Romero, 2001) and a single measure of plasma CORT concentrations may be difficult to interpret. In that respect, measuring feather CORT concentrations is promising because it provides eco-toxicologists not only with a reliable indicator of trace element contamination (Burger, 1993; Lodenius and Solonen, 2013), but also with an integrated measure of CORT secretion (Bortolotti et al., 2008; Jenni-Eiermann et al., 2015). Thanks to this methodology, here, we report for the first time strong and significant correlations between feather Cd and Pb concentrations and feather CORT levels in both juvenile and adult blackbirds. Previous biomedical studies have shown that long-term exposure to low doses of Pb can affect the

HPA axis, and thus CORT secretion, in laboratory rodents and humans (Haider et al., 2013; Virgolini et al., 2005). Although Pb exposure has been associated with alteration of behavior and physiological functions in wild vertebrates (Burger and Gochfeld, 2005; Chatelain et al., 2016; Geens et al., 2009; Martinez-Haro et al., 2011), only a few studies have examined the impact of Pb exposure on CORT levels (Eeva et al., 2003, 2014). For example, Baos et al. (2006) found that Pb exposure was associated with elevated maximum CORT levels in white storks (*Ciconia ciconia*), supporting therefore the idea that Pb exposure may affect the HPA axis and CORT regulation. Similarly, although few studies have investigated the effect of Cd exposure on CORT levels, there is also evidence of an effect of Cd on CORT secretion from both experimental (Di Giulio and Scanlon, 1984) and field studies in birds (Strong et al., 2015; Wayland et al., 2002).

Finally, we also found that feather CORT concentrations were significantly and positively correlated with Hg burden in blackbirds. Although the impact of Hg contamination has been overlooked in terrestrial birds (Jackson et al., 2015), a few previous studies have reported an impact of such contamination on CORT levels in wild vertebrates, but they reported either reduced or unchanged CORT levels in contaminated animals (Beck et al., 2014; Franceschini et al., 2009; Heath et al., 2005; Herring et al., 2012; Tartu et al., 2015; Wada et al., 2009, 2010). Although further experimental studies appear now necessary, the positive relationship between Hg and CORT concentrations in adult blackbirds may result from independent effects of urbanization on Hg concentrations and CORT secretion without any direct effect of Hg on CORT levels.

Because this study is correlative, it is obviously challenging to assess the exact impact of Cd, Pb and Hg contamination on CORT regulation. All three of these metals could act as endocrine disruptors and affect the functioning of the HPA axis, explaining therefore the higher feather CORT levels of urban birds. On the other hand, both CORT secretion and non-essential trace element concentrations may be affected by urbanization without any functional link between these two variables. Therefore, future experimental studies are now needed to understand to what extent Cd, Hg and Pb may disrupt CORT secretion, and more generally physiological mechanisms, in terrestrial birds (Jackson et al., 2015). Moreover, as our study was based on a limited geographical area and sample size, future studies should now explore these questions at a large geographical scale to fully assess the influence of urbanization on trace element concentrations and their relationships with stress physiology in wild vertebrates.

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References

Alleva, E., Francia, N., Pandolfi, M., De Marinis, A.M., Chiarotti, F., Santucci, D., 2006. Organochlorine and heavy-metal contaminants in wild mammals and birds of Urbino-Pesaro province, Italy: an analytic overview for potential bioindicators. Arch. Environ. Contam. Toxicol. 51, 123–134.

Angelier, F., Wingfield, J.C., 2013. Importance of the glucocorticoid stress response in a changing world: Theory, hypotheses and perspectives. Gen. Comp. Endocrinol. 190, 118–128.

Angelier, F., Wingfield, J.C., Parenteau, C., Pellé, M., Chastel, O., 2015. Does short-term fasting lead to stressed-out parents? A study of incubation commitment and the hormonal stress responses and recoveries in snow petrels. Horm. Behav. 67, 28–37.

Azimi, S., Ludwig, A., Thévenot, D.R., Colin, J.-L., 2003. Trace metal determination in total atmospheric deposition in rural and urban areas. Sci. Total Environ. 308, 247–256.

Azimi, S., Rocher, V., Muller, M., Moilleron, R., Thevenot, D.R., 2005. Sources, distribution and variability of hydrocarbons and metals in atmospheric deposition in an urban area (Paris, France). Sci. Total Environ. 337, 223–239.

Baos, R., Bias, J., Bortolotti, G.R., Marchant, T.A., Hiraldo, F., 2006. Adrenocortical response to stress and thyroid hormone status in free-living nestling white storks (Ciconia ciconia) exposed to heavy metal and arsenic contamination. Environ. Health Perspect. 114, 1497–1501.

Beck, M.L., Hopkins, W.A., Hallagan, J.J., Jackson, B.P., Hawley, D.M., 2014. Exposure to residual concentrations of elements from a remediated coal fly ash spill does not adversely influence stress and immune responses of nestling tree swallows. Conserv. Physiol. 2, cou018.

Berglund, A.M.M., Koivula, M.J., Eeva, T., 2011. Species- and age-related variation in metal exposure and accumulation of two passerine bird species. Environ. Pollut. 159, 2368–2374.

Bianchi, N., Ancora, S., Di Fazio, N., Leonzio, C., 2008. Cadmium, lead, and mercury levels in feathers of small passerine birds: Noninvasive sampling strategy. Environ. Toxicol. Chem. 27, 2064–2070.

Bichet, C., Scheifler, R., Cøeurdassier, M., Julliard, R., Sorci, G., Loiseau, C., 2013. Urbanization, trace metal pollution, and malaria prevalence in the house sparrow. PLoS One 8, e53866.

Blévin, P., Carravieri, A., Jaeger, A., Chastel, O., Bustamante, P., Cherel, Y., 2013. Wide range of mercury contamination in chicks of Southern Ocean seabirds. PLoS One 8, e54508.

Bonier, F., 2012. Hormones in the city: Endocrine ecology of urban birds. Horm. Behav. 61, 763–772.

Bortolotti, G.R., Marchant, T.A., Blas, J., German, T., 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct. Ecol. 22, 494–500.

Brasso, R.L., Cristol, D.A., 2008. Effects of mercury exposure on the reproductive success of tree swallows (Tachycineta bicolor). Ecotoxicology 17, 133–141.

Burger, J., 1993. Metals in avian feathers: bioindicators of environmental pollution. Rev. Environ. Toxicol. 5, 203–311.

Burger, J., Bowman, R., Woolfenden, G.E., Gochfeld, M., 2004. Metal and metalloid concentrations in the eggs of threatened Florida scrub-jays in suburban habitat from south-central Florida. Sci. Total Environ. 328, 185–193.

Burger, J., Gochfeld, M., 2005. Effects of lead on learning in herring gulls: an avian wildlife model for neurobehavioral deficits. Neurotoxicology 26, 615–624.

Burger, J., Gochfeld, M., 2004. Marine birds as sentinels of environmental pollution. EcoHealth 1, 263–274.

Burger, J., Gochfeld, M., 1992. Trace element distribution in growing feathers: Additional excretion in feather sheaths. Arch. Environ. Contam. Toxicol. 23, 105–108.

Carlson, J.R., Cristol, D., Swaddle, J.P., 2014. Dietary mercury exposure causes decreased escape takeoff flight performance and increased molt rate in European starlings (Sturnus vulgaris). Ecotoxicology 23, 1464–1473.

Carravieri, A., Bustamante, P., Tartu, S., Meillère, A., Labadie, P., Budzinski, H., Peluhet, L., Barbraud, C., Weimerskirch, H., Chastel, O., others, 2014. Wandering albatrosses document latitudinal variations in the transfer of persistent organic pollutants and mercury to Southern Ocean predators. Environ. Sci. Technol. 48, 14746–14755.

Carson, R., 1962. Silent spring. Houghton Mifflin, Boston.

Chadwick, E.A., Simpson, V.R., Nicholls, A.E., Slater, F.M., 2011. Lead levels in Eurasian otters decline with time and reveal interactions between sources, prevailing weather, and stream chemistry. Environ. Sci. Technol. 45, 1911–1916.

Chalmers, A.T., Krabbenhoft, D.P., Van Metre, P.C., Nilles, M.A., 2014. Effects of urbanization on mercury deposition and accumulation in New England. Environ. Pollut. 192, 104–112.

Coeurdassier, M., Fritsch, C., Faivre, B., Crini, N., Scheifler, R., 2012. Partitioning of Cd and Pb in the blood of European blackbirds (Turdus merula) from a smelter contaminated site and use for biomonitoring. Chemosphere 87, 1368–1373.

Colborn, T., 2004. Neurodevelopment and endocrine disruption. Environ. Health Perspect. 112, 944–949.

Colborn, T., vom Saal, F.S., Soto, A.M., 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101, 378.

Cramp, S., 1988. Handbook of the birds of Europe and the Middle East and North Africa. Volume V - Tyrant flycatchers to thrushes. Oxford University Press, Oxford.

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R., Eens, M., 2000. Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? Arch. Environ. Contam. Toxicol. 39, 541–546.

Dauwe, T., Bervoets, L., Pinxten, R., Blust, R., Eens, M., 2003. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. Environ. Pollut. 124, 429–436.

Dauwe, T., Janssens, E., Bervoets, L., Blust, R., Eens, M., 2004. Relationships between metal concentrations in great tit nestlings and their environment and food. Environ. Pollut. 131, 373–380.

Dauwe, T., Janssens, E., Eens, M., 2006. Effects of heavy metal exposure on the condition and health of adult great tits (Parus major). Environ. Pollut. 140, 71–78.

Desrochers, A., 1992. Age and foraging success in European blackbirds: variation between and with individuals. Anim. Behav. 43, 885–894.

Domingo, J.L., 1994. Metal–induced developmental toxicity in mammals. J. Toxicol. Environ. Health 42, 123–141.

Eens, M., Pinxten, R., Verheyen, R.F., Blust, R., Bervoets, L., 1999. Great and blue tits as indicators of heavy metal contamination in terrestrial ecosystems. Ecotoxicol. Environ. Saf. 44, 81–85.

Eeva, T., Hasselquist, D., Langefors, Å., Tummeleht, L., Nikinmaa, M., Ilmonen, P., 2005. Pollution related effects on immune function and stress in a free-living population of pied flycatcher Ficedula hypoleuca. J. Avian Biol. 36, 405–412.

Eeva, T., Lehikoinen, E., Nikinmaa, M., 2003. Pollution-induced nutritional stress in birds: an experimental study of direct and indirect effects. Ecol. Appl. 13, 1242–1249.

Eeva, T., Rainio, M., Berglund, A., Kanerva, M., Stauffer, J., Stöwe, M., Ruuskanen, S., 2014. Experimental manipulation of dietary lead levels in great tit nestlings: limited effects on growth, physiology and survival. Ecotoxicology 23, 914–928.

Eeva, T., Tanhuanpää, S., Råbergh, C., Airaksinen, S., Nikinmaa, M., Lehikoinen, E., 2000. Biomarkers and fluctuating asymmetry as indicators of pollution-induced stress in two hole-nesting passerines. Funct. Ecol. 14, 235–243.

Evans, K.L., Hatchwell, B.J., Parnell, M., Gaston, K.J., 2010. A conceptual framework for the colonisation of urban areas: the blackbird Turdus merula as a case study. Biol. Rev. 85, 643–667.

Evans, K.L., Newton, J., Gaston, K.J., Sharp, S.P., McGowan, A., Hatchwell, B.J., 2012. Colonisation of urban environments is associated with reduced migratory behaviour, facilitating divergence from ancestral populations. Oikos 121, 634–640.

Evers, D.C., Savoy, L.J., DeSorbo, C.R., Yates, D.E., Hanson, W., Taylor, K.M., Siegel, L.S., Cooley Jr, J.H., Bank, M.S., Major, A., others, 2008. Adverse effects from environmental mercury loads on breeding common loons. Ecotoxicology 17, 69–81.

Franceschini, M.D., Lane, O.P., Evers, D.C., Reed, J.M., Hoskins, B., Romero, L.M., 2009. The corticosterone stress response and mercury contamination in free-living tree swallows, Tachycineta bicolor. Ecotoxicology 18, 514–521.

Frantz, A., Pottier, M.-A., Karimi, B., Corbel, H., Aubry, E., Haussy, C., Gasparini, J., Castrec-Rouelle, M., 2012. Contrasting levels of heavy metals in the feathers of urban pigeons from close habitats suggest limited movements at a restricted scale. Environ. Pollut. 168, 23–28.

Frederick, P., Jayasena, N., 2010. Altered pairing behaviour and reproductive success in white ibises exposed to environmentally relevant concentrations of methylmercury. Proc. R. Soc. B rspb20102189. doi:doi:10.1098/rspb.2010.2189

Furness, R.W., 1993. Birds as monitors of pollutants, in: Furness, R.W., Greenwood, J.J.D. (Eds.), Birds as Monitors of Environmental Change. Chapman & Hall, London, pp. 86–143.

Furness, R.W., Camphuysen, K.C., 1997. Seabirds as monitors of the marine environment. ICES J. Mar. Sci. J. Cons. 54, 726–737.

Gasparini, J., Jacquin, L., Laroucau, K., Vorimore, F., Aubry, E., Castrec-Rouëlle, M., Frantz, A., 2014. Relationships Between Metals Exposure and Epidemiological Parameters of Two Pathogens in Urban Pigeons. Bull. Environ. Contam. Toxicol. 92, 208–212.

Geens, A., Dauwe, T., Bervoets, L., Blust, R., Eens, M., 2010. Haematological status of wintering great tits (Parus major) along a metal pollution gradient. Sci. Total Environ. 408, 1174–1179.

Geens, A., Dauwe, T., Eens, M., 2009. Does anthropogenic metal pollution affect carotenoid colouration, antioxidative capacity and physiological condition of great tits (Parus major)? Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 150, 155–163.

Giesy, J.P., Feyk, L.A., Jones, P.D., Kannan, K., Sanderson, T., 2003. Review of the effects of endocrine-disrupting chemicals in birds. Pure Appl. Chem. 75, 2287–2303.

Goutte, A., Bustamante, P., Barbraud, C., Delord, K., Weimerskirch, H., Chastel, O., 2014. Demographic responses to mercury exposure in two closely related Antarctic top predators. Ecology 95, 1075–1086.

Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X., Briggs, J.M., 2008. Global change and the ecology of cities. Science 319, 756–760.

Haider, S., Saleem, S., Tabassum, S., Khaliq, S., Shamim, S., Batool, Z., Parveen, T., Haleem, D.J., others, 2013. Alteration in plasma corticosterone levels following long term oral administration of lead produces depression like symptoms in rats. Metab. Brain Dis. 28, 85–92.

Hallinger, K.K., Cornell, K.L., Brasso, R.L., Cristol, D.A., 2011. Mercury exposure and survival in free-living tree swallows (Tachycineta bicolor). Ecotoxicology 20, 39–46.

Hawley, D.M., Hallinger, K.K., Cristol, D.A., 2009. Compromised immune competence in free-living tree swallows exposed to mercury. Ecotoxicology 18, 499–503.

Heath, J.A., Frederick, P.C., Karasov, W.H., 2005. Relationships among mercury concentrations, hormones, and nesting effort of white ibises (Eudocimus albus) in the Florida Everglades. The Auk 122, 255–267.

Hernández, L.M., Gómara, B., Fernández, M., Jiménez, B., González, M.J., Baos, R., Hiraldo, F., Ferrer, M., Benito, V., Suner, M.A., others, 1999. Accumulation of heavy metals and As in wetland birds in the area around Donana National Park affected by the Aznalcollar toxic spill. Sci. Total Environ. 242, 293–308.

Herring, G., Ackerman, J.T., Herzog, M.P., 2012. Mercury exposure may suppress baseline corticosterone levels in juvenile birds. Environ. Sci. Technol. 46, 6339–6346.

Herring, G., Eagles-Smith, C.A., Gawlik, D.E., Beerens, J.M., Ackerman, J.T., 2014. Physiological condition of juvenile wading birds in relation to multiple landscape stressors in the Florida Everglades: effects of hydrology, prey availability, and mercury bioaccumulation. PLoS One 9, e106447.

Hoff Brait, C.H., Antoniosi Filho, N.R., 2011. Use of feathers of feral pigeons (Columba livia) as a technique for metal quantification and environmental monitoring. Environ. Monit. Assess. 179, 457–467.

Isaksson, C., 2010. Pollution and its impact on wild animals: a meta-analysis on oxidative stress. EcoHealth 7, 342–350.

Jackson, A.K., Evers, D.C., Adams, E.M., Cristol, D.A., Eagles-Smith, C., Edmonds, S.T., Gray, C.E., Hoskins, B., Lane, O.P., Sauer, A., others, 2015. Songbirds as sentinels of mercury in terrestrial habitats of eastern North America. Ecotoxicology 24, 453–467.

Jager, L.P., Rijnierse, F.V., Esselink, H., Baars, A.J., 1996. Biomonitoring with the Buzzard Buteo buteo in the Netherlands: heavy metals and sources of variation. J. Für Ornithol. 137, 295–318.

Janssens, E., Dauwe, T., Bervoets, L., Eens, M., 2002. Inter- and intraclutch variability in heavy metals in feathers of great tit nestlings (Parus major) along a pollution gradient. Arch. Environ. Contam. Toxicol. 43, 323–329.

Janssens, E., Dauwe, T., Van Duyse, E., Beernaert, J., Pinxten, R., Eens, M., 2003. Effects of heavy metal exposure on aggressive behavior in a small territorial songbird. Arch. Environ. Contam. Toxicol. 45, 121–127.

Jenni-Eiermann, S., Helfenstein, F., Vallat, A., Glauser, G., Jenni, L., 2015. Corticosterone: effects on feather quality and deposition into feathers. Methods Ecol. Evol. 6, 237–246.

Kahle, S., Becker, P.H., 1999. Bird blood as bioindicator for mercury in the environment. Chemosphere 39, 2451–2457.

Kalisińska, E., Lisowski, P., Salicki, W., Kucharska, T., Kavetska, K., 2009. Mercury in wild terrestrial carnivorous mammals from north-western Poland and unusual fish diet of red fox. Acta Theriol. (Warsz.) 54, 345–356.

Kalisińska, E., Salicki, W., Mys\lek, P., Kavetska, K.M., Jackowski, A., 2004. Using the mallard to biomonitor heavy metal contamination of wetlands in North-Western Poland. Sci. Total Environ. 320, 145–161.

Kekkonen, J., Hanski, I.K., Väisänen, R.A., Brommer, J.E., 2012. Levels of heavy metals in House Sparrows (Passer domesticus) from urban and rural habitats of southern Finland. Ornis Fenn. 89, 91–98.

Kitaysky, A.S., Kitaiskaia, E.V., Wingfield, J.C., Piatt, J.F., 2001. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. J. Comp. Physiol. B 171, 701–709.

Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 1999. Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. Funct. Ecol. 13, 577–584.

Koivula, M.J., Kanerva, M., Salminen, J.-P., Nikinmaa, M., Eeva, T., 2011. Metal pollution indirectly increases oxidative stress in great tit (Parus major) nestlings. Environ. Res. 111, 362–370.

Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen. Comp. Endocrinol. 148, 132–149.

Lodenius, M., Solonen, T., 2013. The use of feathers of birds of prey as indicators of metal pollution. Ecotoxicology 22, 1319–1334.

Lormée, H., Jouventin, P., Trouve, C., Chastel, O., 2003. Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies Sula sula. Ibis 145, 212–219.

Lynn, S.E., Stamplis, T.B., Barrington, W.T., Weida, N., Hudak, C.A., 2010. Food, stress, and reproduction: short-term fasting alters endocrine physiology and reproductive behavior in the zebra finch. Horm. Behav. 58, 214–222.

Magrath, R.D., 1991. Nestling weight and juvenile survival in the blackbird, Turdus merula. J. Anim. Ecol. 60, 335–351.

Manjula, M., Mohanraj, R., Devi, M.P., 2015. Biomonitoring of heavy metals in feathers of eleven common bird species in urban and rural environments of Tiruchirappalli, India. Environ. Monit. Assess. 187, 1–10.

Martinez-Haro, M., Green, A.J., Mateo, R., 2011. Effects of lead exposure on oxidative stress biomarkers and plasma biochemistry in waterbirds in the field. Environ. Res. 111, 530–538.

Marzluff, J.M., Bowman, R., Donnelly, R., 2001. A historical perspective on urban bird research: trends, terms, and approaches, in: Marzluff, J.M., Bowman, R., Donnelly, R. (Eds.), Avian Ecology and Conservation in an Urbanizing World. Springer, pp. 1–17.

McEwen, B.S., Wingfield, J.C., 2010. What's in a name? Integrating homeostasis, allostasis and stress. Horm. Behav. 57, 105–111.

Meillère, A., Brischoux, F., Parenteau, C., Angelier, F., 2015. Influence of Urbanization on Body Size, Condition, and Physiology in an Urban Exploiter: A Multi-Component Approach. PLoS One 10, e0135685.

Mergler, D., Anderson, H.A., Chan, L.H.M., Mahaffey, K.R., Murray, M., Sakamoto, M., Stern, A.H., 2007. Methylmercury exposure and health effects in humans: a worldwide concern. Ambio 36, 3–11.

Metian, M., Bustamante, P., Hédouin, L., Warnau, M., 2008. Accumulation of nine metals and one metalloid in the tropical scallop Comptopallium radula from coral reefs in New Caledonia. Environ. Pollut. 152, 543–552.

Møller, A.P., Diaz, M., Flensted-Jensen, E., Grim, T., Ibáñez-Álamo, J.D., Jokimäki, J., Mänd, R., Markó, G., Tryjanowski, P., 2012. High urban population density of birds reflects their timing of urbanization. Oecologia 170, 867–875.

Møller, A.P., Jokimäki, J., Skorka, P., Tryjanowski, P., 2014. Loss of migration and urbanization in birds: a case study of the blackbird (Turdus merula). Oecologia 175, 1019–1027.

Nam, D.-H., Lee, D.-P., 2006. Monitoring for Pb and Cd pollution using feral pigeons in rural, urban, and industrial environments of Korea. Sci. Total Environ. 357, 288–295.

Nriagu, J.O., 1990. Human influence on the global cycling of trace metals. Glob. Planet. Change 2, 113–120.

Nriagu, J.O., 1989. A global assessment of natural sources of atmospheric trace metals. Nature 338, 47–49.

Orlowski, G., Kasprzykowski, Z., Dobicki, W., Pokorny, P., Polechoński, R., 2010. Geographical and habitat differences in concentrations of copper, zinc and arsenic in eggshells of the Rook Corvus frugilegus in Poland. J. Ornithol. 151, 279–286.

Ottinger, M.A., Quinn, M.J., Lavoie, E., Abdelnabi, M.A., Thompson, N., Hazelton, J.L., Wu, J.M., Beavers, J., Jaber, M., 2005. Consequences of endocrine disrupting chemicals on reproductive endocrine function in birds: establishing reliable end points of exposure. Domest. Anim. Endocrinol. 29, 411–419.

Pacyna, J.M., Pacyna, E.G., 2001. An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. Environ. Rev. 9, 269–298.

Paradis, E., Baillie, S.R., Sutherland, W.J., Gregory, R.D., 1998. Patterns of natal and breeding dispersal in birds. J. Anim. Ecol. 67, 518–536.

Partecke, J., Gwinner, E., 2007. Increased sedentariness in European Blackbirds following urbanization: a consequence of local adaptation? Ecology 88, 882–890.

Partecke, J., Schwabl, I., Gwinner, E., 2006. Stress and the city: urbanization and its effects on the stress physiology in European blackbirds. Ecology 87, 1945–1952.

Peakall, D., 1992. Animal biomarkers as pollution indicators. Springer Science & Business Media.

Pouyat, R.V., Szlavecz, K., Yesilonis, I.D., Wong, C.P., Murawski, L., Marra, P., Casey, R.E., Lev, S., 2015. Multi-scale assessment of metal contamination in residential soil and soil fauna: a case study in the Baltimore–Washington metropolitan region, USA. Landsc. Urban Plan. 142, 7–17.

R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Rice, K.M., Walker Jr, E.M., Wu, M., Gillette, C., Blough, E.R., 2014. Environmental mercury and its toxic effects. J. Prev. Med. Pub. Health 47, 74.

Rimmer, C.C., Miller, E.K., McFarland, K.P., Taylor, R.J., Faccio, S.D., 2010. Mercury bioaccumulation and trophic transfer in the terrestrial food web of a montane forest. Ecotoxicology 19, 697–709.

Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. 19, 249–255.

Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model—a new model integrating homeostasis, allostasis, and stress. Horm. Behav. 55, 375–389.

Roux, K.E., Marra, P.P., 2007. The presence and impact of environmental lead in passerine birds along an urban to rural land use gradient. Arch. Environ. Contam. Toxicol. 53, 261–268.

Scheifler, R., Coeurdassier, M., Morilhat, C., Bernard, N., Faivre, B., Flicoteaux, P., Giraudoux, P., Noel, M., Piotte, P., Rieffel, D., de Vaufleury, A., Badot, P.-M., 2006. Lead concentrations in feathers and blood of common blackbirds (Turdus merula) and in earthworms inhabiting unpolluted and moderately polluted urban areas. Sci. Total Environ. 371, 197–205.

Scheuhammer, A.M., 1987. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: a review. Environ. Pollut. 46, 263–295.

Scheuhammer, A.M., Meyer, M.W., Sandheinrich, M.B., Murray, M.W., 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36, 12–19.

Selin, N.E., 2009. Global biogeochemical cycling of mercury: a review. Annu. Rev. Environ. Resour. 34, 43.

Snoeijs, T., Dauwe, T., Pinxten, R., Vandesande, F., Eens, M., 2004. Heavy metal exposure affects the humoral immune response in a free-living small songbird, the great tit (Parus major). Arch. Environ. Contam. Toxicol. 46, 399–404.

Streif, M., Rasa, O., 2001. Divorce and its consequences in the common blackbird Turdus merula. Ibis 143, 554–560.

Swaileh, K.M., Sansur, R., 2006. Monitoring urban heavy metal pollution using the House Sparrow (Passer domesticus). J. Environ. Monit. 8, 209–213.

Tan, S.W., Meiller, J.C., Mahaffey, K.R., 2009. The endocrine effects of mercury in humans and wildlife. Crit. Rev. Toxicol. 39, 228–269.

Tartu, S., Angelier, F., Wingfield, J.C., Bustamante, P., Labadie, P., Budzinski, H., Weimerskirch, H., Bustnes, J.O., Chastel, O., 2015. Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. Sci. Total Environ. 505, 180–188.

Tartu, S., Goutte, A., Bustamante, P., Angelier, F., Moe, B., Clément-Chastel, C., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2013. To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biol. Lett. 9, 20130317.

United Nations, 2015. World Urbanization Prospects: The 2014 Revision. Department of Economic and Social Affairs, Population Division, United Nations, New York.

Van der Gon, H.D., van het Bolscher, M., Visschedijk, A., Zandveld, P., 2007. Emissions of persistent organic pollutants and eight candidate POPs from UNECE–Europe in 2000, 2010 and 2020 and the emission reduction resulting from the implementation of the UNECE POP protocol. Atmos. Environ. 41, 9245–9261.

Varian-Ramos, C.W., Swaddle, J.P., Cristol, D.A., 2014. Mercury reduces avian reproductive success and imposes selection: an experimental study with adult-or lifetime-exposure in zebra finch. PloS One 9, e95674.

Virgolini, M.B., Chen, K., Weston, D.D., Bauter, M.R., Cory-Slechta, D.A., 2005. Interactions of chronic lead exposure and intermittent stress: consequences for brain catecholamine systems and associated behaviors and HPA axis function. Toxicol. Sci. 87, 469–482.

Wada, H., Cristol, D.A., McNabb, F.A., Hopkins, W.A., 2009. Suppressed adrenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. Environ. Sci. Technol. 43, 6031–6038.

Wada, H., Yates, D.E., Evers, D.C., Taylor, R.J., Hopkins, W.A., 2010. Tissue mercury concentrations and adrenocortical responses of female big brown bats (Eptesicus fuscus) near a contaminated river. Ecotoxicology 19, 1277–1284.

Walker, C.H., 2003. Neurotoxic pesticides and behavioural effects upon birds. Ecotoxicology 12, 307–316.

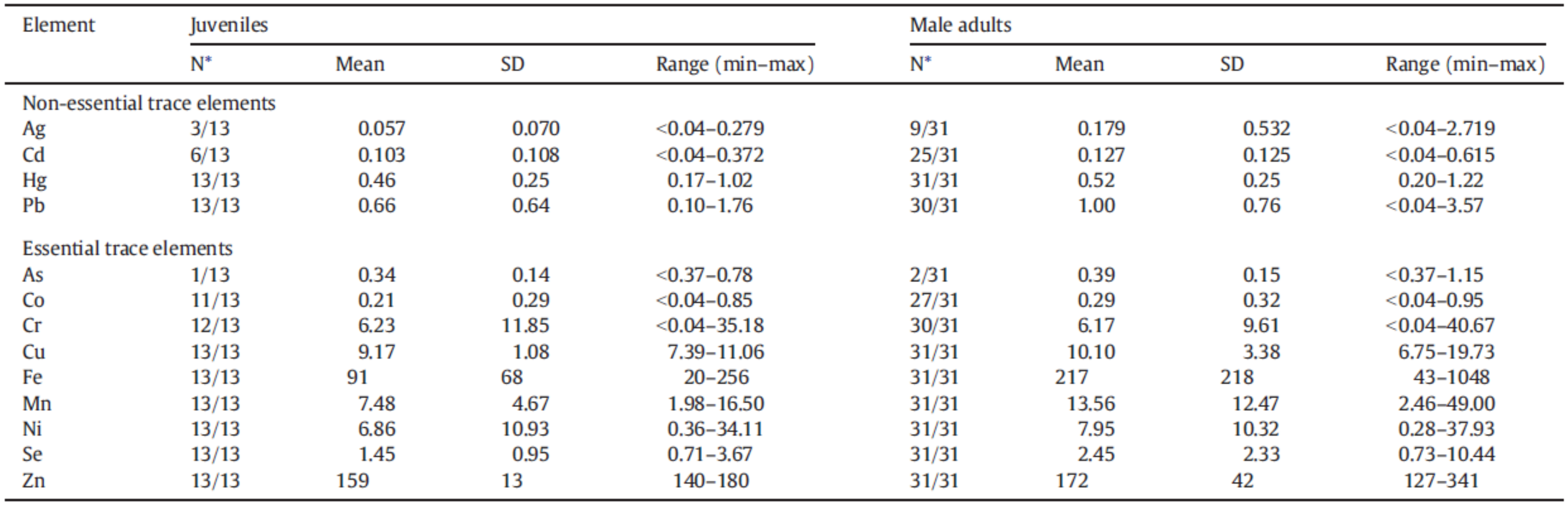
Walker, C.H., Sibly, R.M., Hopkin, S.P., Peakall, D.B., 2012. Principles of Ecotoxicology, Fourth Edition. ed. CRC Press, Taylor & Francis Group.

Wei, B., Yang, L., 2010. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. Microchem. J. 94, 99–107.

Wingfield, J.C., 2008. Comparative endocrinology, environment and global change. Gen. Comp. Endocrinol. 157, 207–216.

Wolfe, M.F., Schwarzbach, S., Sulaiman, R.A., 1998. Effects of mercury on wildlife: a comprehensive review. Environ. Toxicol. Chem. 17, 146–160.

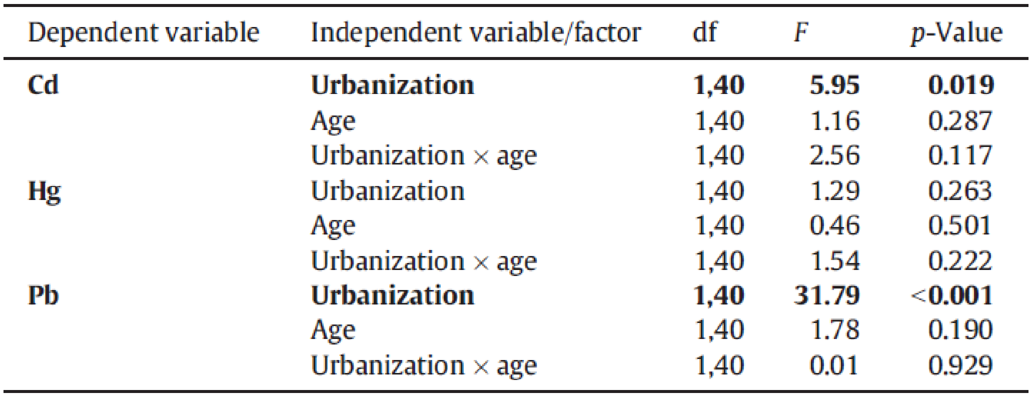
Table 1. Trace elements concentrations (µg.g^{-1} dw) in breast feathers of juvenile and male adult



Element	Juveniles				Male adults			
	N*	Mean	SD	Range (min–max)	N*	Mean	SD	Range (min–max)
Non-essential trace elements								
Ag	3/13	0.057	0.070	<0.04–0.279	9/31	0.179	0.532	<0.04–2.719
Cd	6/13	0.103	0.108	<0.04–0.372	25/31	0.127	0.125	<0.04–0.615
Hg	13/13	0.46	0.25	0.17–1.02	31/31	0.52	0.25	0.20–1.22
Pb	13/13	0.66	0.64	0.10–1.76	30/31	1.00	0.76	<0.04–3.57
Essential trace elements								
As	1/13	0.34	0.14	<0.37–0.78	2/31	0.39	0.15	<0.37–1.15
Co	11/13	0.21	0.29	<0.04–0.85	27/31	0.29	0.32	<0.04–0.95
Cr	12/13	6.23	11.85	<0.04–35.18	30/31	6.17	9.61	<0.04–40.67
Cu	13/13	9.17	1.08	7.39–11.06	31/31	10.10	3.38	6.75–19.73
Fe	13/13	91	68	20–256	31/31	217	218	43–1048
Mn	13/13	7.48	4.67	1.98–16.50	31/31	13.56	12.47	2.46–49.00
Ni	13/13	6.86	10.93	0.36–34.11	31/31	7.95	10.32	0.28–37.93
Se	13/13	1.45	0.95	0.71–3.67	31/31	2.45	2.33	0.73–10.44
Zn	13/13	159	13	140–180	31/31	172	42	127–341

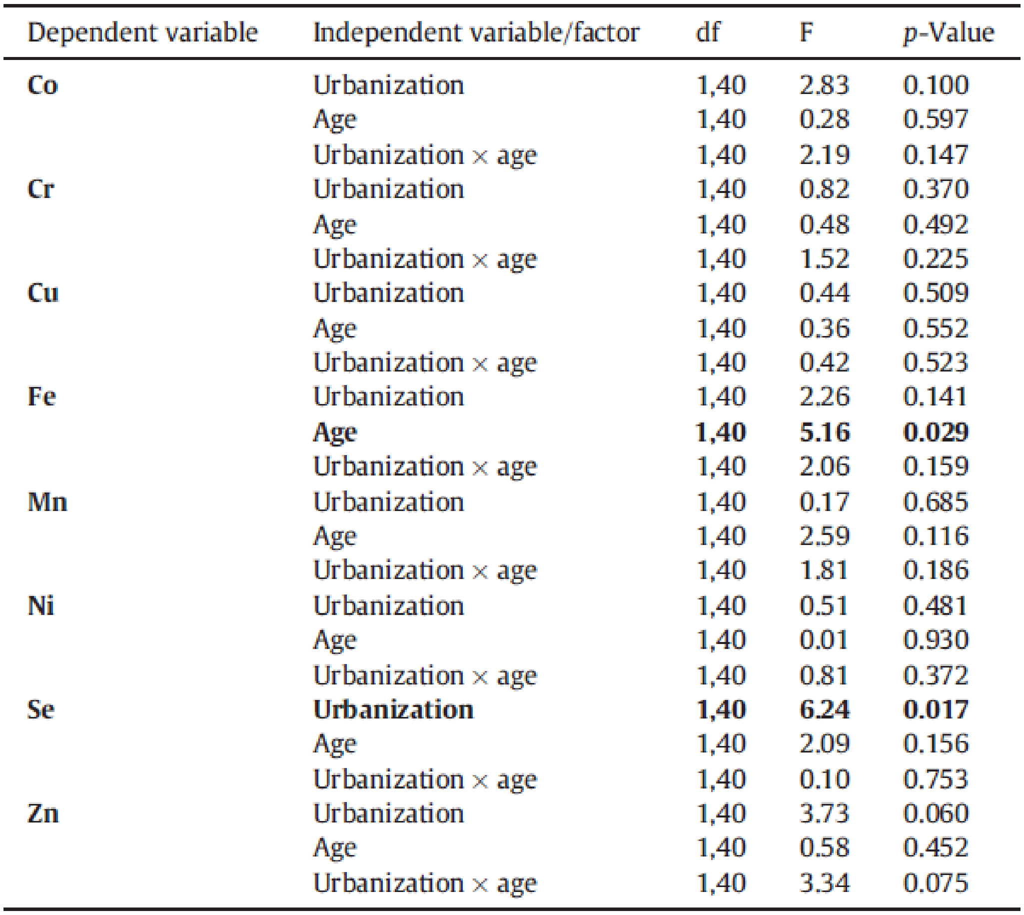
* N: sample with concentration above the LoD /sample size.

Table 2. Linear models investigating the influence of urbanization, age (juvenile vs. adult) and their interaction on feather non-essential trace element (Cd, Hg and Pb) concentrations. Significant variables ($p < 0.05$) are shown in bold.



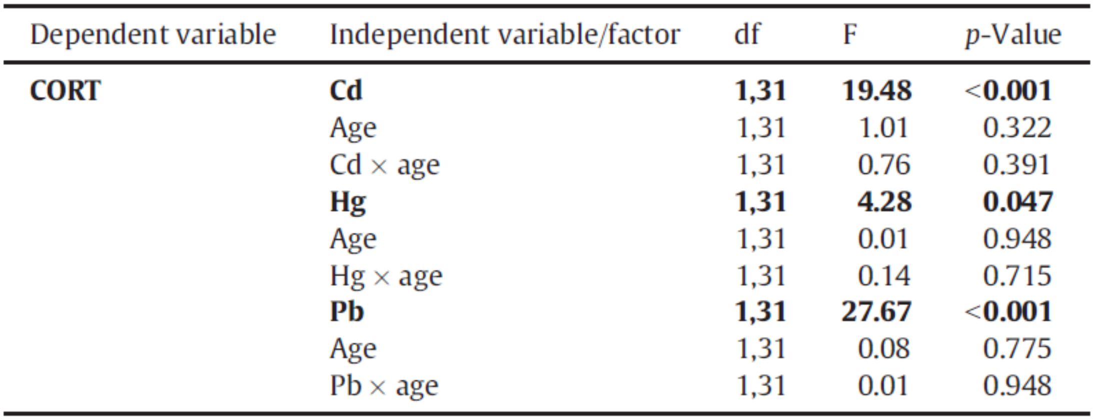
Dependent variable	Independent variable/factor	df	*F*	*p*-Value
Cd	**Urbanization**	**1,40**	**5.95**	**0.019**
	Age	1,40	1.16	0.287
	Urbanization × age	1,40	2.56	0.117
Hg	Urbanization	1,40	1.29	0.263
	Age	1,40	0.46	0.501
	Urbanization × age	1,40	1.54	0.222
Pb	**Urbanization**	**1,40**	**31.79**	<**0.001**
	Age	1,40	1.78	0.190
	Urbanization × age	1,40	0.01	0.929

Table3. Linear models investigating the influence of urbanization, age (juvenile vs. adult) and their interaction on feather essential trace element (Co, Cr, Cu, Fe, Mn, Ni, Se and Zn) concentrations. Significant variables ($p < 0.05$) are shown in bold.



Dependent variable	Independent variable/factor	df	F	*p*-Value
Co	Urbanization	1,40	2.83	0.100
	Age	1,40	0.28	0.597
	Urbanization × age	1,40	2.19	0.147
Cr	Urbanization	1,40	0.82	0.370
	Age	1,40	0.48	0.492
	Urbanization × age	1,40	1.52	0.225
Cu	Urbanization	1,40	0.44	0.509
	Age	1,40	0.36	0.552
	Urbanization × age	1,40	0.42	0.523
Fe	Urbanization	1,40	2.26	0.141
	Age	**1,40**	**5.16**	**0.029**
	Urbanization × age	1,40	2.06	0.159
Mn	Urbanization	1,40	0.17	0.685
	Age	1,40	2.59	0.116
	Urbanization × age	1,40	1.81	0.186
Ni	Urbanization	1,40	0.51	0.481
	Age	1,40	0.01	0.930
	Urbanization × age	1,40	0.81	0.372
Se	**Urbanization**	**1,40**	**6.24**	**0.017**
	Age	1,40	2.09	0.156
	Urbanization × age	1,40	0.10	0.753
Zn	Urbanization	1,40	3.73	0.060
	Age	1,40	0.58	0.452
	Urbanization × age	1,40	3.34	0.075

Table 4. Linear models investigating the relationships between non-essential trace elements and CORT concentrations. All models also include age (juvenile vs. adult) as a covariate and the "trace element × age" interaction. Significant variables ($p < 0.05$) are shown in bold.



Dependent variable	Independent variable/factor	df	F	*p*-Value
CORT	**Cd**	**1,31**	**19.48**	<**0.001**
	Age	1,31	1.01	0.322
	Cd × age	1,31	0.76	0.391
	Hg	**1,31**	**4.28**	**0.047**
	Age	1,31	0.01	0.948
	Hg × age	1,31	0.14	0.715
	Pb	**1,31**	**27.67**	<**0.001**
	Age	1,31	0.08	0.775
	Pb × age	1,31	0.01	0.948

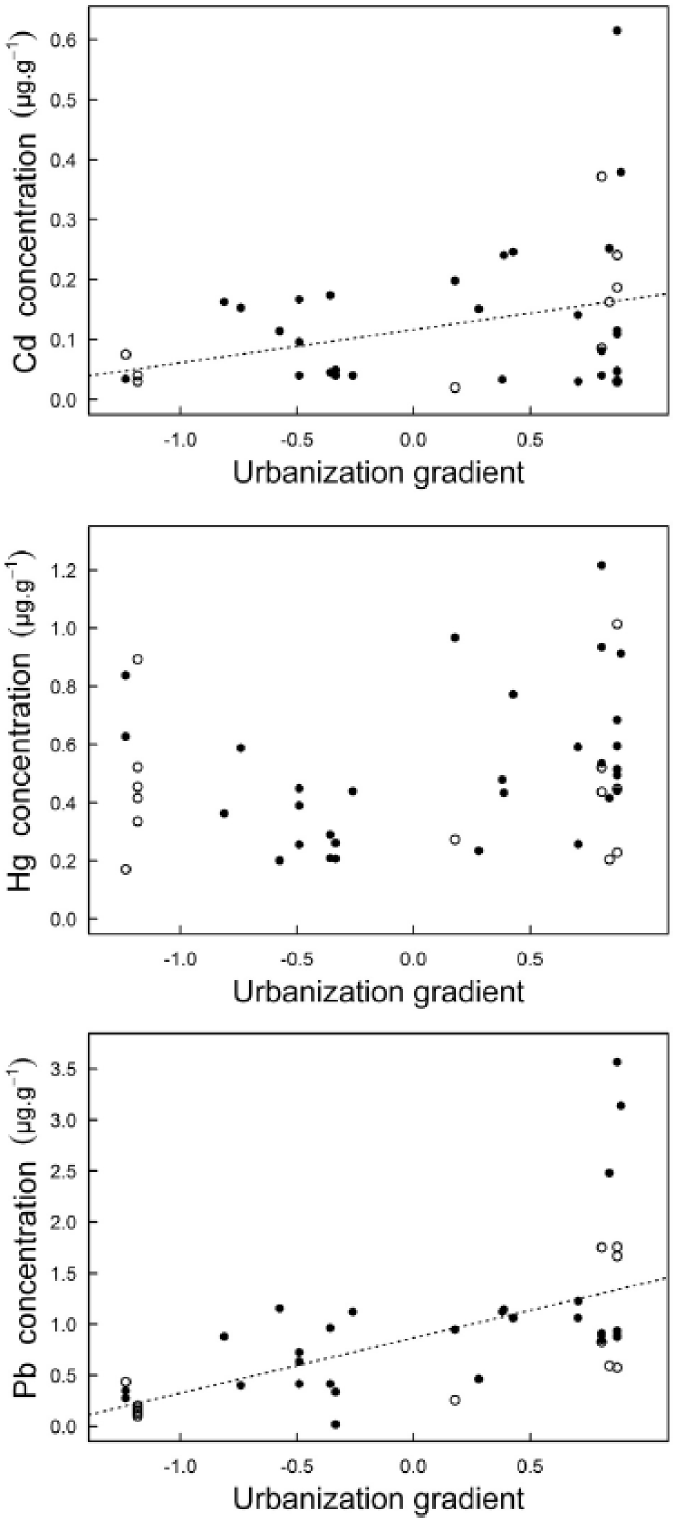


Fig. 1. Non-essential element (Cd, Hg, and Pb) concentrations (μg·g−1 dw) in breast feathers of juvenile (open circles) and adult male (filled circles) blackbirds sampled along an urbanization gradient. Dotted lines refer to statistically significant linear regressions.

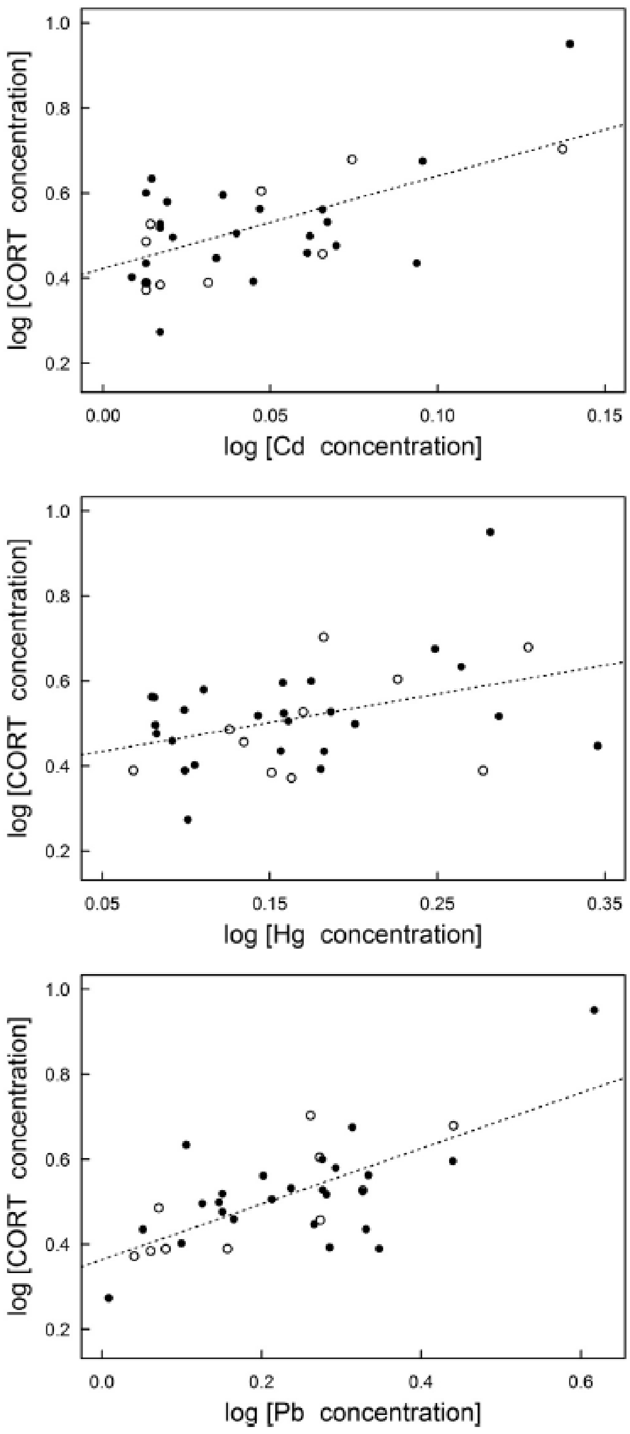


Fig 2. Relationships between feather non-essential trace element concentrations (Cd, Hg, Pb, μg·g^{-1}; log-transformed) and feather CORT concentrations (ng·mm−1; log transformed) in blackbirds. Open circles represent juvenile and filled circles represent adult male individuals. Dotted lines refer to statistically significant linear regressions. Importantly, results were similar when analyses were performed after removal of the individual with a very high CORT level (see text for details).

Supplementary Information

Corticosterone levels in relation to trace element contamination along an urbanization gradient in the Common blackbird (*Turdus merula*)

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Table S1: Habitat characteristics of the sampling location and sample sizes. Locations are listed in decreasing order of urbanization (PC1 values from a principal component analysis conducted on the five variables of land cover).

		Habitat characteristics (km^2)						
Coordinates	**Type**	**Urban areas**	**Arable lands**	**Pastures**	**Woodlands**	**Scrublands**	**Urbanization score (PC1)**	**Sample size**
46°18’16”N;0°27’56”W	Urban	18.59	11.97	7.16	0.76	0.00	0.890	1
46°18’38”N;0°28’30”W	Urban	20.19	10.01	7.25	1.04	0.00	0.873	9
46°10’09”N;1°07’34”W	Urban	25.14	11.69	0.22	0.50	0.93	0.840	2
46°19’49”N;0°27’43”W	Urban	23.97	10.79	2.67	1.05	0.00	0.807	5
46°17’50”N;0°25’31”W	Urban	12.24	23.06	2.93	0.26	0.00	0.707	1
46°20’22”N;0°28’55”W	Urban	17.35	16.83	2.77	1.53	0.00	0.705	1
46°10’49”N;0°15’49”W	Rural	1.04	30.54	6.90	0.00	0.00	0.427	1
46°08’27”N;0°13’13”W	Rural	1.37	34.95	2.17	0.00	0.00	0.388	1
46°14’16”N;0°24’06”W	Rural	1.82	28.13	7.13	0.65	0.75	0.381	1
46°07’23”N;0°07’54”W	Rural	1.77	34.73	1.85	0.00	0.14	0.280	1
46°07’38”N;0°16’05”W	Rural	0.62	28.91	7.89	0.69	0.37	0.178	2
46°07’16”N;0°20’45”W	Rural	0.72	23.89	3.71	6.78	3.38	-0.261	1
46°02’59”N;0°25’43”W	Rural	0.41	29.25	2.48	5.89	0.45	-0.334	2
46°05’53”N;0°20’43”W	Rural	0.72	26.30	3.04	7.72	0.70	-0.357	2
46°06’37”N;0°20’44”W	Rural	0.86	27.00	4.05	5.72	0.85	-0.490	1
46°05’30”N;0°21’45”W	Rural	0.53	24.26	2.86	9.73	1.11	-0.490	2
46°07’05”N;0°23’03”W	Rural	0.70	12.86	2.52	17.47	4.93	-0.574	1
46°04’00”N;0°24’51”W	Rural	0.41	25.38	2.26	8.00	2.45	-0.740	1
46°08’57”N;0°27’49”W	Rural	0.38	25.23	0.48	7.32	5.06	-0.811	1
46°08’49”N;0°25’32”W	Rural	0.00	10.88	0.00	19.72	7.89	-1.183	5
46°08’55”N;0°24’23”W	Rural	0.00	7.28	0.00	22.61	8.59	-1.234	3